U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-294 861

Determination of Halogenated Anilines and Related Compounds by HPLC with Electrochemical and UV Detection

(U.S.) Health Effects Research Lab, Research Triangle Park, NC

16 May 78

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-78-103	2. JOURNAL ARTICLE
3. R	PB 294861
4. TITLE AND SUBTITLE: DETERMINATION OF HALOGENATED ANILINES AND RELATED COMPOUNDS BY HPLC WITH ELECTROCHEMICAL AND UV DETECTION	5. REPORT DATE
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): E. M. Lores, D. W. Bristol, and R. F. Moseman	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Environmental Toxicology Division, Health Effects Research Laboratory, Office of Research and Development, Research Triangle Park, NC 27711	10. PROGRAM ELEMENT NO. 1EA615
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Health Effects Research Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

Published in: Journal of Chromatographic Science 16:358-362, 1978

16. ABSTRACT

Halogenated anilines may enter the environment from a variety of sources, including metabolism of insecticides, herbicides, and fungicides; industrial waste; and reduction of nitrosubstituted aromatic compounds. The toxicity of anilines requires that these compounds be monitored in the environment. Previous methods using gas chromatography required derivatization and were unable to separate some of the halogenated metabolites. An HPLC method has been developed that allows separation and detection of sub-nanogram quantities without derivatization. The separation of these compounds was accomplished on a commercially available C-18 bonded phase column. Sub-nanogram quantities were detected using an inexpensive electrochemical detector. A faster, but less sensitive UV method was also developed. Chromatograms and sensitivity data for the various compounds are presented. The applications and limitations of the electrochemical detector are discussed.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
CHEMICAL ANALYSIS PESTICIDES DETECTORS	HIGH PRESSURE LIQUID CHROMATOGRAPHY	07, C

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)*: UNCLASSIFIED	21. NO. OF PAGES: 7
	20. SECURITY CLASS *(This page)*: UNCLASSIFIED	22. PRICE: PC A02-MF A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-78-103
JOURNAL ARTICLE

Determination of Halogenated Anilines and Related Compounds by HPLC with Electrochemical and UV Detection

E.M. Lores, D.W. Bristol*, and R.F. Moseman, Analytical Chemistry Branch, Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Abstract

Halogenated anilines may enter the environment from a variety of sources, including metabolism of insecticides, herbicides, and fungicides; industrial waste; and reduction of nitrosubstituted aromatic compounds. The toxicity of anilines requires that these compounds be monitored in the environment. Previous methods using gas chromatography required derivatization and were unable to separate some of the halogenated metabolites. An HPLC method has been developed that allows separation and detection of sub-nanogram quantities without derivatization. The separation of these compounds was accomplished on a commercially available C-18 bonded phase column. Sub-nanogram quantities were detected using an inexpensive electrochemical detector. A faster, but less sensitive UV method was also developed. Chromatograms and sensitivity data for the various compounds are presented. The applications and limitations of the electrochemical detector are discussed.

Introduction

The need to develop improved methods for measuring toxic chemicals in the environment and the development of new detectors (1) and columns for high pressure liquid chromatography (HPLC) have made possible the development of a new and sensitive method for the analysis of halogenated anilines. These toxicants may enter the environment from several sources. Industrial discharge, pesticide degradation and transformation, dyes, and combustion of plastics and urethane products are but a few of the pathways. One of the most common of these pathways is pesticide degradation and transformation. Carbamates, ureas, and anilides can be metabolized or degraded in the environment to yield halogenated anilines. In many cases, the transformation products from these pesticides are more toxic than the parent compounds. A list of the halogenated anilines and some of the parent pesticides used in this study is shown in Table I.

The polar nature of substituted anilines makes analysis by gas chromatography (GC) difficult without first derivatizing the compounds. Even after derivatization, Bradway and Shafik (2) found it difficult to separate some isomers by GC.

Table I. Possible Origins of Various Anilines

#	Metabolite	Origin	Type Pesticide
I	aniline	Propham	Carbamate
		Carbetamide	Carbamate
		Fenuron	Urea
		Siduron	Urea
II	2-amino-4-chlorophenol	Barban	Carbamate
III	*o*-chloro-aniline	Drazoxolone	-isoxalone
		Dyrene	Triazine
IV	*o*-bromo-aniline	Metabromuron	Urea
V	*m*-chloro-aniline	Chloropropham	Carbamate
		Barban	Carbamate
VI	*p*-chloro-aniline	Monuron	Urea
		Monolinuron	Urea
		Urox	Urea
		Dimilin	Urea
VII	3,4-dichloro-aniline	Propanil	Anilide
		Diuron	Urea
		Linuron	Urea

An HPLC method was developed in this study that not only separates a complex mixture of halogenated anilines without derivatization but also is sensitive enough to detect sub-nanogram quantities of most of the anilines tested.

This method uses a C-18 bonded phase column and a mobile phase consisting of mixtures of phosphate buffer at pH 2.1 and acetonitrile for separation of the anilines. A commercially available electrochemical system is used for

*U.S. Food and Drug Administration, Division of Chemistry & Physics, (HFF 147) 200 C St., Washington, DC 20204.

detection of small quantities of anilines. A UV detector and gradient elution can be used if the extra sensitivity is not required. Since gradient elution cannot be used with the electrochemical detector, two different elution mixtures were developed to achieve complete separation of the experimental mixture studied. The selectivity and sensitivity of the electrochemical detector should make this method useful for assessing human exposure and for environmental monitoring.

Equipment and Materials

Mobile Phase Solvents - Pesticide or UV quality acetonitrile was used throughout the study. The 0.15 M phosphate buffer was prepared daily at a pH of 2.1.

Standards - All standards were obtained as technical or practical grade materials and were distilled or recrystallized before use. The melting points or boiling points were determined and agreed with literature values.

HPLC Systems - Two HPLC systems were used in this study: A Waters HPLC with dual Model 6000 A pumps, a Model 660 solvent programmer, a Model 440 absorbance detector operated at 254 nm, and a Model U6K injector; and a Tracor Model 995 isochromatographic pump with an LC-2A electrochemical detector (available from Bioanalytical Systems, Inc., West Lafayette, IN) and a Valco 7000 psi injector.

Column - A 15 cm Zorbax® C-18 column gave the best results in this study.

Scanning Cyclic Voltameter - A scanning cyclic voltameter (Bioanalytical Systems, Inc.) was used to determine the oxidation potentials of each of the compounds.

Experimental *

HPLC of Aniline Mixture Utilizing UV Detection

For the detection of quantities ranging from 10 ng to several μg, a UV detector (λ254) was used. The separation of all seven anilines was accomplished by programming the mobile phase from 80% buffer/20% acetonitrile to 40% buffer/60% acetonitrile at 10%/min. The flow rate was maintained at 2 ml/min. The mobile phase was changed to 100% acetonitrile for overnight column storage. Since the use of more than 70% acetonitrile caused precipitation of salts that plugged the column, it was necessary to change from buffer to distilled water to flush the column before programming to 100% acetonitrile.

Chromatography of Anilines for Electrochemical Detection

Since the electrochemical detector is extremely sensitive to any change in parameters, solvent programming was not possible. Thus, two different solvent systems were required for separation of the seven anilines. Compounds I, II, and III were chromatographed with a mobile phase of 80% buffer/20% acetonitrile and a flow rate 1 ml/min. For the separation of later eluting anilines (compounds IV, V, VI, and VII) the mobile phase was changed to 60% buffer/40% acetonitrile and the flow rate increased to 2 ml/min. The separation of these compounds into the two groups is somewhat arbitrary and considerable flexibility in the group assignment is possible. The detector oxidation potential was maintained at +1.1V throughout the experiment.

Results and Discussion

UV detection and solvent programming offer a rapid means of analysis for halogenated anilines when the selectivity and sensitivity are not required. As can be seen in Figure 1, the complete analysis of all seven compounds requires less than 6 minutes. The speed of separation and simplicity of the UV detector make this combination very useful for analyzing quantities greater than 10 ng.

The electrochemical detector offers a sensitive and somewhat selective means of detection for HPLC (3). There are, however, several problems associated with this system. The detector cannot be used with flow or solvent programming. A waiting period is required for any changes that are made: i.e., applied voltage, solvent composition, flow rate, or initial start-up each day. These waiting periods vary from 10 min for minor changes in flow to several hours for daily start-up when operating at maximum sensitivity. A waiting period of about 30 min is required for changing solvent systems.

Two different solvent systems are required with the electrochemical detector because no single solvent system would permit the elution of all the anilines in a reasonable time with sufficient separation. Chromatograms obtained from these two solvent systems used with the electrochemical detector are shown in Figures 2 and 3. These chromatograms

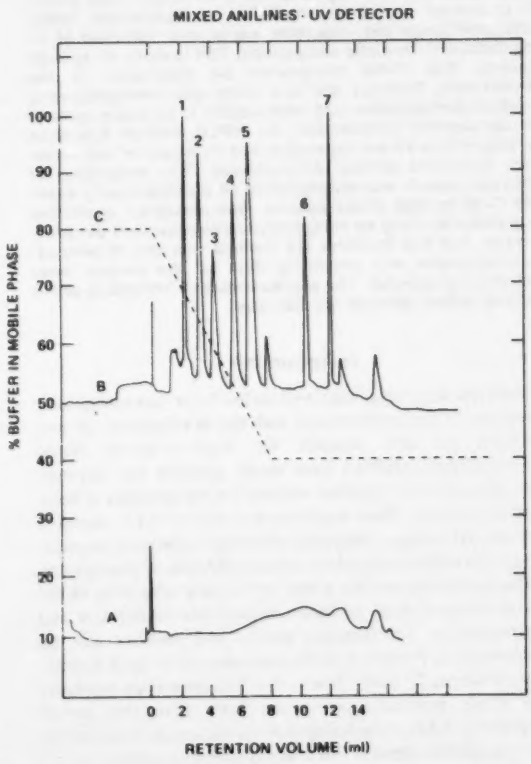

Figure 1. Chromatograms obtained using a 15 cm Zorbax C-18 column with solvent programming and UV detection which show: (A) a blank profile run; (B) a chromatogram of a mixture of standards containing (1) 40 ng aniline; (2) 70 ng 2-amino-5-chlorophenol; (3) 50 ng *p*-chloroaniline; (4) 75 ng *p*-bromoaniline; (5) 60 ng *m*-chloroaniline; (6) 90 ng 2-chloroaniline; (7) 180 ng 3,4-dichloroaniline; (C) the gradient profile showing the percent buffer in the mobile phase.

* Use of trade names is for identification purposes only and does not consitute endorsement by the U.S. Environmental Protection Agency.

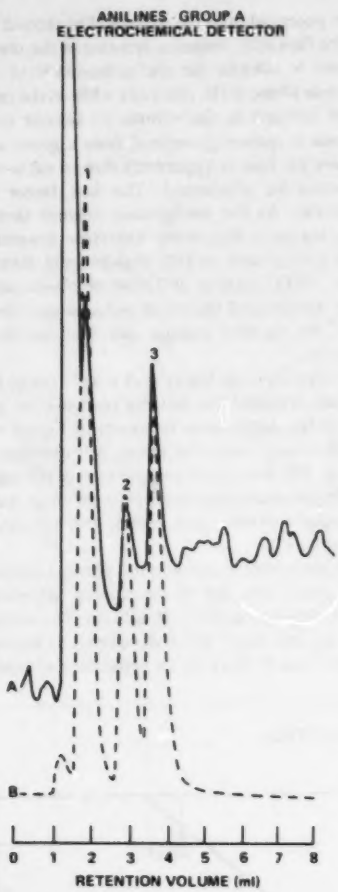

Figure 2. Chromatograms of the anilines in "group A" obtained using an electrochemical detector which show: **A** (1) .52 ng aniline; (2) .32 ng 2-amino-5-chlorophenol; (3) .64 ng *p*-chloroaniline; **B** (1) 65 ng aniline; (2) 40 ng 2-amino-5-chlorophenol; (3) 80 ng *p*-chloroaniline.

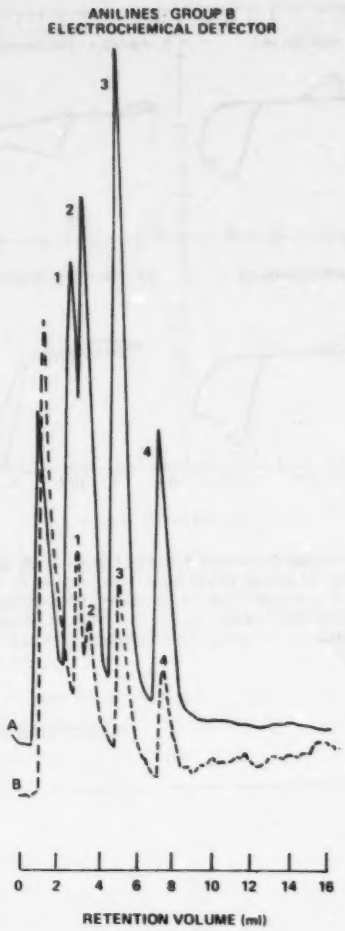

Figure 3. Chromatograms of the "group B" anilines obtained using an electrochemical detector which show: **A** (1) 15 ng *p*-bromoaniline; (2) 10.7 ng *m*-chloroaniline; (3) 18.7 ng *o*-chloroaniline; (4) 12.5 ng 3,4-dichloroaniline; **B** (1) 1.2 ng *p*-bromoaniline; (2) 0.86 ng *m*-chloroaniline; (3) 1.5 ng *o*-chloroaniline; (4) 1 ng 3,4-dichloroaniline.

were obtained using the same column that was used in Figure 1. The percent acetonitrile and the maximum flow rate that can be used are limited by the electrochemical detector. Buffer solutions containing greater than 50% acetonitrile do not provide enough electrolyte for proper performance of the detector. Use of quaternary ammonium salts allows higher concentrations of acetonitrile in the mobile phase. Flow rates greater than 2.5 ml/min can erode the surface of the carbon paste electrode.

The limit of detection for each of the compounds is shown in Table II. This table is based on a quantity which gives a signal/noise ratio≥2 using the conditions given in this paper. Changes in k' or the detector potential setting will change these values. As can be seen in Table II the sensitivity of the detector is more than adequate for most applications.

There are several factors that affect sensitivity of the detector: applied voltage, flow rate, volume injected, and background current. The first factor, applied voltage, can have very dramatic effects on the sensitivity. The voltage can be used to "tune" certain compounds in or out. This can be very helpful when compounds cannot be separated and the compound of interest has a lower oxidation potential. A scanning cyclic voltamogram can be useful for determining the oxidation potential. Cyclic voltamograms of several of the anilines are shown in Figure 4. Note that the phenol is oxidized

Table II. Limit of Detection*

Aniline	0.23 ng
2-amino,4-chlorophenol	0.28 ng
p-chloroaniline	0.33 ng
p-bromoaniline	0.38 ng
m-chloroaniline	0.27 ng
o-chloroaniline	0.28 ng
3,4-dichloroaniline	0.38 ng

*Based on the quantity required to give a response that is twice the noise level.

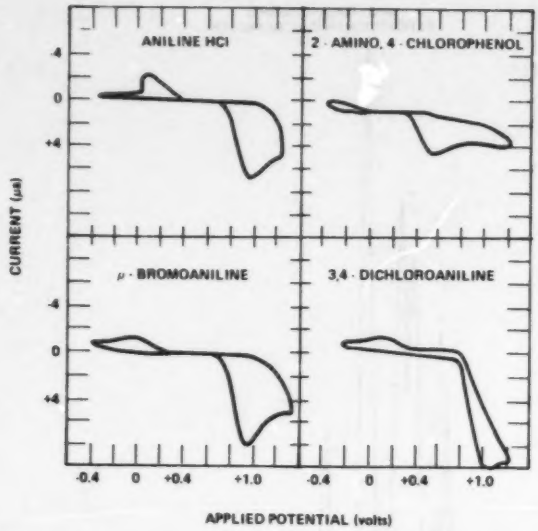

Figure 4. Cyclic voltamograms of some of the anilines. Sweep rate: 50 mV/sec; graphite paste electrode, Ag/AgCl reference electrode. The solvent was 50/50 acetonitrile/phosphate buffer, and the concentration was ~ 0.1 mg/ml. Note the oxidation potential of the aminochlorophenol is much lower than the anilines.

at a much lower potential than the anilines. The second factor, an increase in the flow rate, causes a decrease in the sensitivity because less time is allowed for the compounds to diffuse through the mobile phase to the electrode while in the cell. The third factor, an increase in the volume of sample injected, causes a decrease in current generated from a given amount of sample (Figure 5). This is apparently due to extra-column effects that cannot be eliminated. The last factor is the background current. As the background current increases, the signal to noise ratio decreases, therefore lowering the sensitivity. The background usually results from metal contaminants in the HPLC system. If Teflon or plastic columns can be used the background should be reduced considerably. An increase in the applied voltage can also increase the background current.

The detector appears to be linear over a wide range for the compounds tested. A plot of the detector response *vs.* quantities of several of the compounds is shown in Figure 6. The other compounds tested were also linear, but overlapped on the common plot. The maximum sample size is 100 ng since the injection of larger quantities can form a polymerized coating on the electrode surface and reduce the sensitivity of subsequent injections.

The pH of the phosphate buffer and the percent acetonitrile in the mobile phase are the major factors affecting the separation of the anilines on the C-18 column. The conditions employed here are essentially those developed by Horvath et al. (4) and Graffeo and Karger (5) for separation of ionogenic

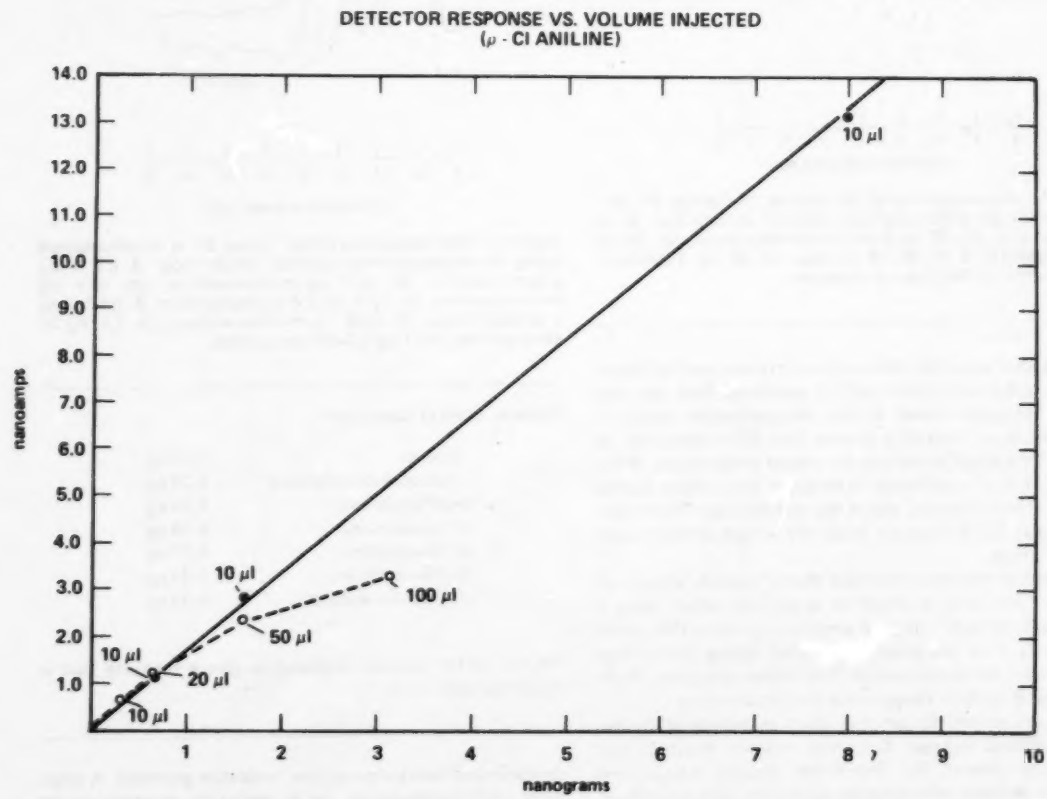

Figure 5. A graph of detector response *vs.* quantity of *p*-chloroaniline injected. The solid line represents a constant volume. The dashed line represents a constant concentration.

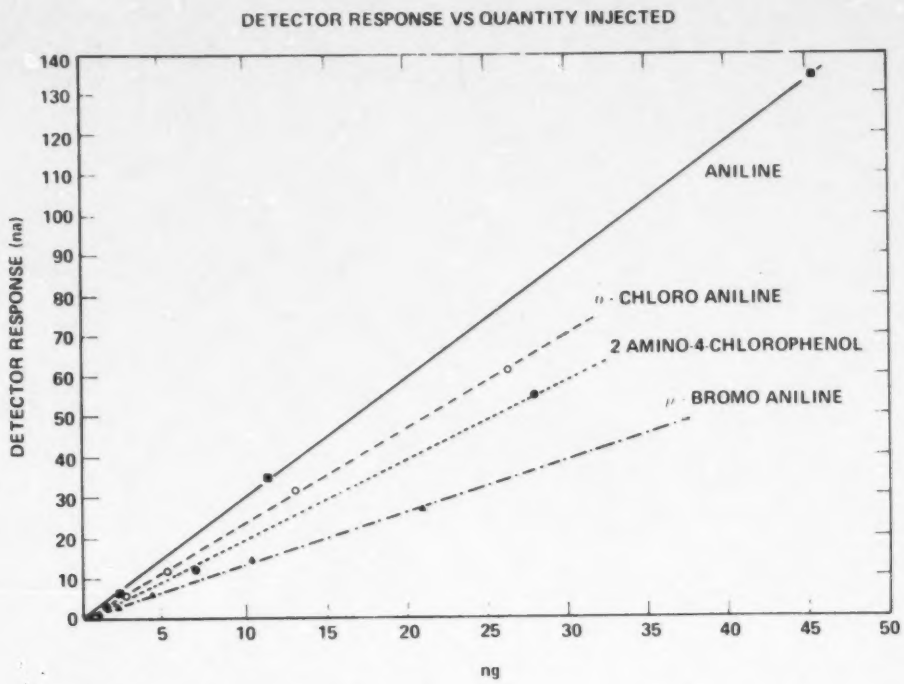

Figure 6. A graph of detector response *vs.* quantity injected (constant volume - 10 µl) for several of the anilines. The other anilines fall within the slopes of those indicated.

solutes including carboxylic acids, phenols, catechol amines, and amino acids. By employing a low pH in this work, the halogenated aniline compounds are separated not as the neutral free bases, but as their corresponding anilinium ions. Therefore, the use of a lower pH buffer will decrease the elution time for all the compounds. But, since the compounds are retained by their reversible association with the hydrocarbon surface of the stationary phase, an increase in the percent acetonitrile in the mobile phase will also reduce the retention time of all the compounds. Knowledge of these factors can be used to increase or decrease separation as needed.

Further studies are in progress to apply this method to the analysis of urine and environmental samples. The emphasis will be to determine if the selectivity of the detector can circumvent interferences or the need for time-consuming cleanup procedures.

Summary

This paper describes a rapid and simple separation of a complex group of halogenated anilines using HPLC with solvent programming and UV detection. Also described is a more sensitive but complex method based on an electrochemical detector. The problems and the factors affecting the sensitivity and selectivity of the electrochemical detector are discussed. New developments in the field of electrochemical detection may eliminate many of the problems. For applications requiring sensitivity and selectivity, the electrochemical detector is most useful, however, for other applications, UV detection is preferred.

References

1. P.T Kissinger, C. Refshauge, R. Dreiling, and R.N. Adams. An electrochemical detector for liquid chromatography with picogram sensitivity. *Anal. Lett.* 6: 465 (1973).
2. D.E. Bradway and T. Shafik. Electron capture gas chromatographic analysis of the amine metabolites of pesticides. *J. Chromatogr. Sci.* 15: 322-28 (1977).
3. P.T. Kissinger. Amperometric and coulometric detectors for high-performance liquid chromatography. *Anal. Chem.* 49: 477-56 (1977).
4. C. Horvath, W. Melander, and I. Molnar. Liquid chromatography of ionogenic substances with nonpolar stationary phases. *Anal. Chem.* 49: 142-53 (1977).
5. A.P. Graffeo and B.L. Karger. Analysis of indole compounds in urine by high-performance liquid chromatography with fluorometric detection *Clin. Chem.* 22: 184-87 (1976).

Manuscript received; May 16, 1978
revision received June 19, 1978